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## Research



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## Global change biology

## Does sex really matter? Explaining intraspecies variation in ocean acidification responses

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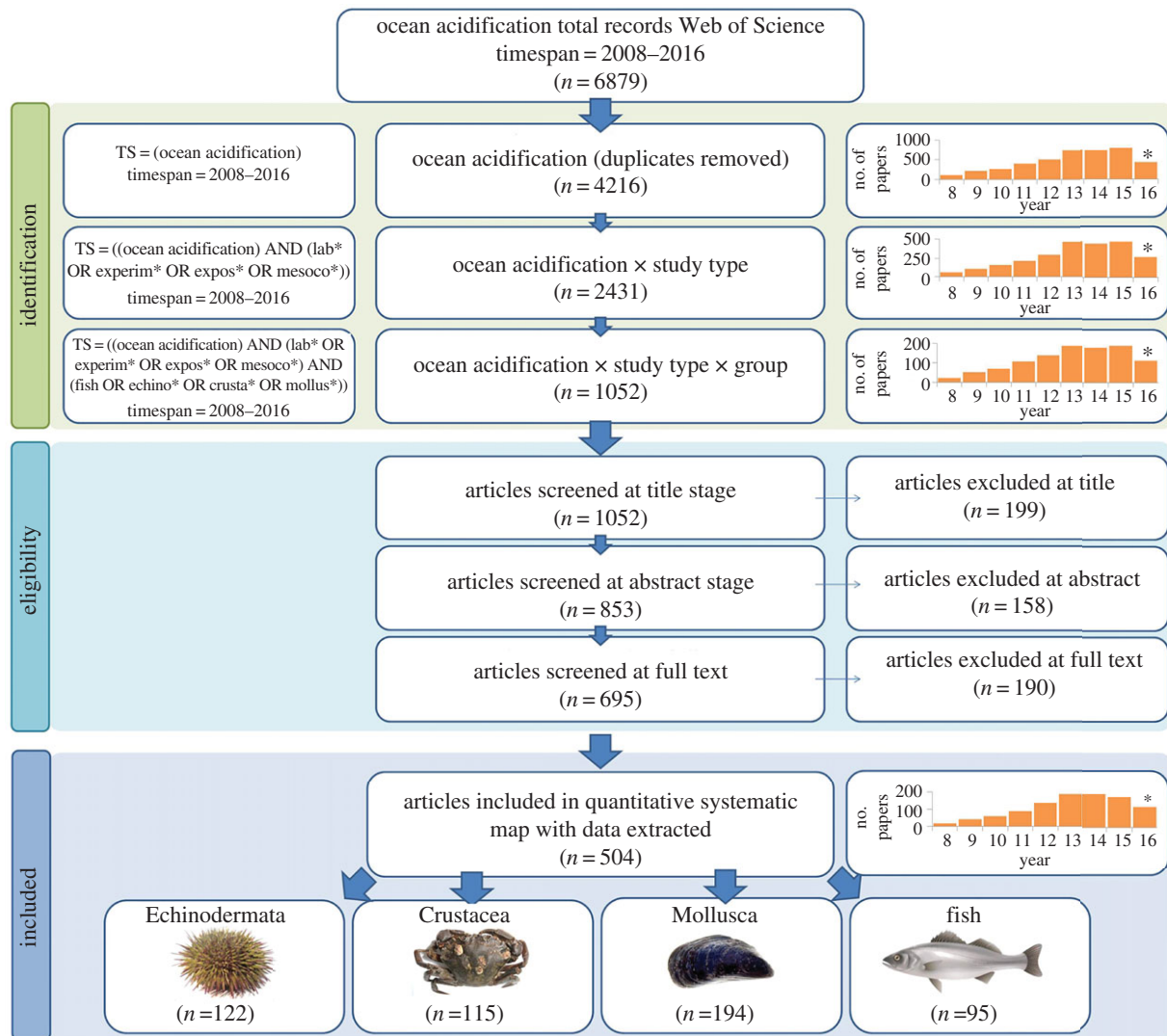
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Ocean acidification (OA) poses a major threat to marine ecosystems globally, having significant ecological and economic importance. The number and complexity of experiments examining the effects of OA has substantially increased over the past decade, in an attempt to address multi-stressor interactions and long-term responses in an increasing range of aquatic organisms. However, differences in the response of males and females to elevated  $p\text{CO}_2$  have been investigated in fewer than 4% of studies to date, often being precluded by the difficulty of determining sex non-destructively, particularly in early life stages. Here we highlight that sex can significantly impact organism responses to OA, differentially affecting physiology, reproduction, biochemistry and ultimately survival. What is more, these impacts do not always conform to ecological theory based on differential resource allocation towards reproduction, which would predict females to be more sensitive to OA owing to the higher production cost of eggs compared with sperm. Therefore, non-sex-specific studies may overlook subtle but ecologically significant differences in the responses of males and females to OA, with consequences for forecasting the fate of natural populations in a near-future ocean.

## 1. Introduction

Ocean acidification (OA), changes in seawater carbonate chemistry induced by oceanic uptake of anthropogenic  $\text{CO}_2$ , is a significant challenge to marine biodiversity [1], as well as to societies and industries reliant on marine living resources [2], globally. Over the past 10 years, studies investigating the ecological effects of OA have increased exponentially [3]. By incorporating the highly dynamic nature of carbonate chemistry in many natural systems [4], as well as multi-stressor interactions [5], and an ever-increasing range of organisms, life-history stages, communities and multiple generations [5], studies have also become increasingly complex. While this effort has contributed to help better explain species tolerance and increase reliability of future change projections, intraspecific variation in OA responses has received insufficient attention, creating uncertainty in reported responses and their interpretation [6].



**Figure 1.** An overview of the systematic map process. Values ( $n = x$ ) are the number of studies at each stage. An asterisk indicates a partial record for the number of papers published in 2016 due to the literature being sourced on 22 June 2016. Fish image Kovalevska/shutterstock.com.

Identifying the sources and consequences of variability in biological responses is pivotal to understanding a population's ability to cope with environmental change [7,8]. However, despite recent evidence that many physiological, behavioural, immunological, molecular and neuro-toxicological functions are influenced by sex-based differences [9,10], and despite many of these same processes being impacted by elevated  $p\text{CO}_2$  [5], the overarching role of sex in determining response to OA remains understudied [11]. Here, we employ a systematic map approach: a transparent, robust and repeatable method to identify and collect relevant literature to answer the question of how sex is considered within experimental OA research [12]. By critically reviewing existing literature, we highlight evidence for, and discuss potential implications of, omitting sex-based variation in species responses.

## 2. Material and methods

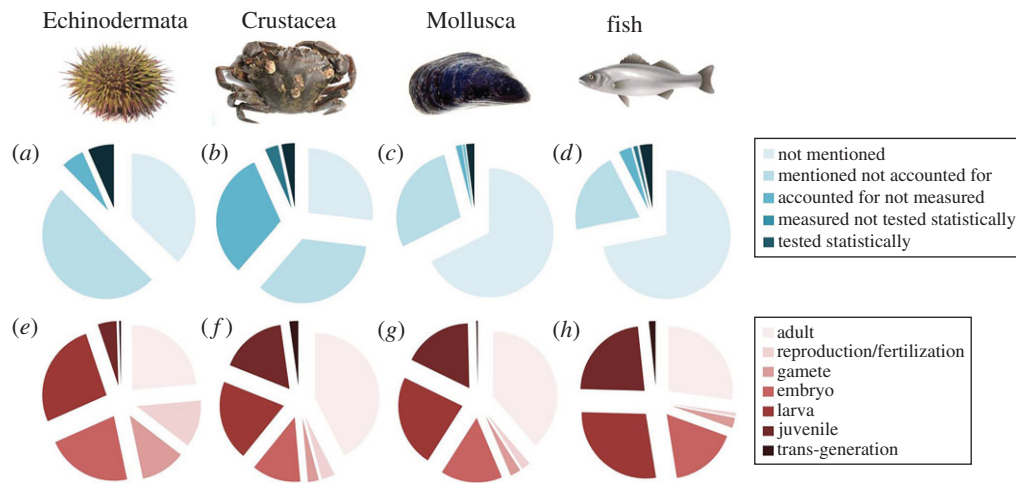
Following international guidelines, a systematic map protocol (electronic supplementary materials) was used to assess existing evidence (figure 1) addressing the research question: *Do OA studies consider the impact of sex on organism responses?* Search term strings using Boolean logic were run through Web of Science to collect relevant peer reviewed literature, and subsequently narrowed to target literature published between January 2008 and May 2016

and limited to studies on fish, crustaceans, echinoderms and molluscs. This ensured a manageable literature set was reviewed while providing a contemporary representation of the OA field. Search results were further refined at three levels to exclude studies irrelevant to our research question (figure 1 and electronic supplementary material). Study inclusion was determined objectively against a set of inclusion criteria, which defined pertinent population, study type, intervention, comparator and outcomes (see electronic supplementary material for details).

Upon inclusion, data on experimental subject (organismal group and species) and life stage (gamete, embryo, larvae, juvenile, adults, as well as transgenerational and reproduction/fertilization processes) were extracted. Each study was then searched for the inclusion of seven sex-related terms within the main body of text (sex, gender, male, female, imposex, intersex or hermaphrodite), and scored according to one of five categories: (i) not mentioned, (ii) mentioned but not accounted for, (iii) accounted for but not measured (e.g. only males used), (iv) measured but not tested statistically, and (v) tested statistically. For the last, endpoints measured and the significance of sex-based differences were extracted.

## 3. Results and discussion

Despite an exponential increase in experimental OA studies over the past decade (figure 1), only 3.9% of these statistically



**Figure 2.** Systematic map results. These show the proportion of studies based on the inclusion of sex as a factor in (a) Echinodermata ( $n = 122$ ), (b) Crustacea ( $n = 115$ ), (c) Mollusca ( $n = 194$ ) and (d) fish ( $n = 95$ ), as well as on the life stage investigated in (e) Echinodermata ( $n = 122$ ), (f) Crustacea ( $n = 115$ ), (g) Mollusca ( $n = 194$ ) and (h) fish ( $n = 95$ ). Fish image Kovalevska/shutterstock.com.

**Table 1.** Overview of the systematic mapping of evidence. Data are pooled across organismal groups ( $n = 504$  articles, figure 1).

classification of sex	no.	%	life stage investigated	no.	%	publication year	no.	%
not mentioned	265	52.58	adult	245	48.61	2008	8	1.59
mentioned not accounted for	168	33.33	reproduction/fertilization	36	7.14	2009	20	3.97
accounted for not measured	53	10.52	gamete	38	7.54	2010	29	5.75
measured not tested statistically	6	1.19	embryo	127	25.20	2011	44	8.73
tested statistically	19	3.77	larva	185	36.71	2012	68	13.49
			juvenile	116	23.02	2013	94	18.65
			trans-generation	8	1.59	2014	94	18.65
						2015	88	17.46
						2016 <sup>a</sup>	58	11.51

<sup>a</sup>Partial record for number of papers published in 2016, as the literature sourced on 22 June 2016.

assessed sex-based differences in OA responses (table 1 and figure 2a–d). Furthermore, only 10.5% of studies account for possible sex effects by assessing males and/or females independently, with over 85% of studies failing to mention or account for sex (table 1). In the majority of instances where tested, sex significantly modified the response of aquatic organisms to OA. This suggests failure to account for sex-based differences could significantly influence the predicted impact of OA on populations.

The relative energetic investment of males and females towards reproduction, in anisogamous systems, is central to the variability observed in organism response to their environment [11]. Consequently, of the studies that differentiated between males and females, around 30% did so by measuring reproductive endpoints. In echinoderms, 6.6% of studies tested for sex-based differences (figure 2a), with reproduction and gamete functionality receiving the greatest attention (figure 2e). Male sea urchins exposed to elevated  $p\text{CO}_2$  and temperature fared worse than females, having significantly lower gonad index and ‘spawnability’ [13,14]. This sex-specific response to OA seems to contradict theory based on projected reproductive strategy. However, gonads in echinoderms are often used as an energy storage compartment that can be filled or depleted depending on conditions [15]. Under OA,

females that invest more in gonadal development may then have access to more energy to cope with stress (e.g. increased costs of acclimation, homeostasis and repair) as compared with males [16]. This outlines the importance of measuring the impacts of OA in both males and females, avoiding overgeneralization and elucidating impact mechanisms by observing organism biology.

A key limitation to investigating male/female differences is the ability to successfully determine sex non-invasively. Sexual dimorphism exists in many adult organisms but in some, including bivalve molluscs, morphological distinction can be unreliable [17], precluding its inclusion experimentally. Consequently, over 96% of studies on the Mollusca neglect to mention or account for sex, the lowest of the four groups investigated (figure 2b), despite Mollusca receiving the greatest attention with respect to OA (figure 1). Conversely, in many adult crustaceans, it is relatively easy to distinguish sex visually, resulting in this group having the greatest percentage of studies that mention or account for sex (63.5%). However, only 3.5% of studies on crustaceans used sex as a factor when performing statistical tests, while 33.9% indirectly accounted for sex by using females or males in isolation (figure 2c).

By pooling data for males and females, or focusing on the response of a single sex, it is possible that species responses

to OA will be inaccurate [18]. This creates an average phenotype, which is a by-product of the mean response of the male and female phenotype, that in reality does not exist. Instead, this creates an artefact on the basis of which we may subsequently make conclusions on how biological systems work [7]. For example, in Crustacea, exposure to elevated  $p\text{CO}_2$  is shown to result in higher mortality in female shrimps (*Palaemon pacificus*) compared with males [19], while the median lethal level ( $\text{LC}_{50}$ ) for  $\text{CO}_2$  is also lower in female copepods (*Acartia tonsa*) compared with males [18]. Sex-specific physiological impacts can result in a twofold increase in the respiration rate of male copepods under elevated  $p\text{CO}_2$ , but respiratory suppression in females [20]. Similarly in molluscs, males and females respond differently to elevated  $p\text{CO}_2$  and temperature, with sex-based differences demonstrated in the mussel (*Mytilus edulis*) metabolome [10] and the biochemical composition of limpet (*Nacella concinna*) gonad [21].

While sex has the potential to impact adult responses to OA, differential mortality of early life stages of males and females exposed to environmental stress clearly has the potential to influence populations [17]. For example, knobbed whelks (*Busycon carica*) show a 1:1 sex ratio in the embryos, and subsequent differential mortality of males and females is proposed as an explanation for a sex ratio often greater than 10:1 in the adult population [22]. However, with respect to OA impacts, sex-based differences in early life stages have largely been restricted to maternal and paternal effects to date, with various protective and inhibitory impacts being shown in transgenerational studies [11]. Inability to non-invasively determine sex in early-life stage individuals has precluded the observation of any sex-based differences in larval OA sensitivity. It is therefore unclear whether sex-based differences are more or less pronounced during early-life stages than in adults [9]. Importantly, any differential mortality, or OA sensitivity, in larval stages could significantly impact the sex ratio of larval recruits, and thus population dynamics [17], as demonstrated in knobbed whelks [22]. In fish, only 3.2% of studies have tested for sex effects (figure 2*d*), likely because the largest proportion of fish studies have investigated larval responses (figure 2*h*).

Sex determination is governed by a diverse suite of different molecular, genetic and environmental factors [17,23]. For example, abiotic conditions (e.g. temperature) are shown to impact sex differentiation and resulting larval condition in fish [24]. Elucidating the possible sex-specific impacts of OA during early-life stages, as well as the impacts of OA on sex determination, is thus key for projecting future population dynamics under climate change scenarios. Furthermore, sex

allocation may change during the lifetime of many marine organisms. Many species of molluscs, crustaceans, annelids and fish exhibit examples of protandrous (mature as male, then change sex to female), protogynous (mature as female, change sex to male), sequential (multiple sex changes during life cycle) or simultaneous (individuals containing both male and female sex organs) hermaphroditism [17]. At a population level, the occurrence of hermaphroditism significantly impacts measured sex ratios. Additionally, where sex-based differences in the response of individuals to OA are shown, any simultaneous hermaphroditism or sex change could alter the perceived response of the population to OA over both a temporal and spatial scale, a factor that has not been considered within the OA literature to date.

Here, we demonstrate that while less than 4% of the OA literature tests for sex-based differences, there is a clear precedent for differential responses to elevated  $p\text{CO}_2$  between sexes. It is possible that studies that have investigated sex-based differences may have done so based on *a priori* reasoning. In doing so, these studies may not offer a representative subsample of the differential responses of males and females to OA. However, the fact that any differences have been demonstrated supports the need for further investigation of this issue. If sex-based differences do exist for economically important species, as seems likely, then capturing this variance is crucial for accurately forecasting the future societal and economic repercussions of OA for dependent sectors, such as coastal management, conservation, fisheries and aquaculture [2]. Unfortunately, the lack of a sufficiently wide evidence base for sex-specific responses currently limits this ambition. As a starting point towards fully elucidating population-level impacts, stronger efforts are needed to consider the influence of sex throughout an organism's life cycle, and its contribution to the variability in species-level responses.

**Data accessibility.** All data are included within the electronic supplementary materials.

**Authors' contributions.** R.P.E. and A.M.Q. conceptualized and designed the study; R.P.E., M.A.U. and W.D. undertook data acquisition, analysis and interpretation; R.P.E. drafted the article. All authors revised the manuscript critically for important intellectual content, approve the final version to be published and agreed to be held accountable for the all aspects of the work.

**Competing interests.** We have no competing interests.

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